failed to affect the channel's total protein level and membrane trafficking. Our data suggest that 14-3-3 is a novel modulator of rEag1 channels in the brain.

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Voltage-Dependent Potassium Channels Kv1.3 and Kv1.5 in Human Cancer
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Membrane ion channels participate in cancerous processes such as proliferation, migration and invasion, which contribute to metastasis. Increasing evidence indicates that voltage-dependent K+ (Kv) channels are involved in the proliferation of many types of cells, including tumor cells. Kv channels have generated immense interest as a promising tool for developing new antitumor therapies. Therefore, the identification of potential biomarkers and therapeutic targets in specific cancers is an important prerequisite for the treatment. Since Kv1.3 and Kv1.5 are involved in the proliferation of many mammalian cells, we aimed to study the expression of Kv1.3 and Kv1.5 in a plethora of human cancers. Thus, tissue from breast, stomach, kidney, bladder, lung, skin, colon, ovary, pancreas, brain, lymph node, skeletal muscle and some of their malignant counterparts have been analyzed. Whereas Kv1.3 expression was either decreased or did not change in most tumors, Kv1.5 was overexpressed. However, the presence of Kv1.3 was mostly associated with inflammatory lymphoplasmocytic cells. Independent of the suitability of individual channels as therapeutic targets, the identification of a Kv phenotype from tumor specimens could have a diagnostic value of its own. Our results demonstrate that Kv1.5, and to some extent Kv1.3, are aberrantly expressed in a number of human cancers. These channels could serve both as novel markers of the metastatic phenotype and as potential new therapeutic targets. The concept of Kv channels as therapeutic targets or prognostic biomarkers attracts increasing interest and warrants further investigation.
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A Model of β1-Adrenergic Regulation of L-Type Ca^{2+} Current and Ryanodine Receptors in Mouse Ventricular Myocytes
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Mathematical modeling protein signaling networks is one of the most rapidly developing fields of science. This includes creation and investigation of the models for protein signaling systems in the heart cells. β_1-adrenergic signaling system is one of the most important systems in cardiac myocytes. We developed an experimentally-based mathematical models of β_1-adrenergic regulation of L-type Ca^{2+} current and ryanodine receptors in mouse ventricular myocytes. The model includes several modules and describes signal transduction in the cells. In the model, β_1-adrenergic receptors (β_1-ARs) are stimulated by application of β_1-adrenergic agonist isoproterenol. Activation of β_1-ARs in turn activates G_s proteins, $G_{s\alpha}$ subunit of which subsequently stimulates cyclic AMP (cAMP) synthesis by adenylyl cyclases. cAMP further activates protein kinase A (PKA) holoenzyme and dissociates PKA catalytic subunit from regulatory subunit. cAMP is degraded by phosphodiesterases (PDEs) that can be inhibited by 3-isobutyl-1-methylxanthine (IBMX). Inhibition of PDEs by IBMX leads to an increase in cAMP level and more profound stimulation of PKA. PKA is also regulated by heat-stable protein kinase inhibitor. β_1-ARs are desensitized by β-adrenergic receptor kinase-1 and by catalytic subunit of PKA that creates negative feedback for this signaling pathway. PKA phosphorylates L-type Ca^{2+} channel, which results in an increase of channel's opening probability and current amplitude. Phosphorylation is removed by protein phosphatases 1 and 2A. We developed a Markov model for L-type Ca^{2+} channel that consists of two parallel activation-inactivation pathways for non-phosphorylated and phosphorylated states. There are also transitions between corresponding non-phosphorylated and phosphorylated states in the model (closed, open, or inactivated states). Similar approach was applied to the Markov model development for ryanodine receptors. Both models reproduced experimentally observed behavior of the channels.

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Modulation of $Ca_v2.2$ Channels via Activation of Human $GABA_B$ Receptors Expressed in HEK293 Cells by Analgesic α-Conotoxins
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Numerous G protein-coupled receptors, including the $GABA_B$ receptor ($GABA_BR$), provide negative feedback to modulate the activity of neuronal (N)-type voltage-gated calcium channels ($Ca_v2.2$), which play a critical role in nociception. We have previously shown that analgesic α-conotoxins indirectly inhibit $Ca_v2.2$ channels via $GABA_BR$ activation in mammalian dorsal root ganglion neurons (1). We reconstituted $GABA_BR$-mediated modulation of stably expressed $Ca_V2.2$ channels (α1, α2δ and β3) in HEK293 cells, co-transfected with cDNAs of cloned human $GABA_BR$ subunits. $GABA_BR$ expression was demonstrated using receptors labelled with fluorescent antibodies against the epitope tags of $GABA_{B1}$ and $GABA_{B2}$ subunits. Modulation of $Ca_v2.2$ by the agonists GABA and baclofen, and the α-conotoxins Vc1.1 and RgIA was studied using the whole-cell recording configuration of the patch clamp technique. Voltage-dependent Ba^{2+} currents were inhibited by baclofen, GABA and α-conotoxins Vc1.1 and RgIA in HEK293 cells transfected with $Ca_v2.2$ and $GABA_BRs$ but not in cells transfected with $Ca_v2.2$ alone. In the presence of the $GABA_BR$, the biophysical properties of the $Ca_v2.2$ channels, including the current-voltage relationship, and activation and steady-state inactivation curves, were unchanged. The concentration-response relationships obtained for inhibition of $Ca_v2.2$ by baclofen, GABA, RgIA, Vc1.1, and cyclized Vc1.1 resulted in half-maximal inhibitory concentrations (IC_{50}) of 3 μM, 68 nM, 130 nM, 120 nM and 10 nM, respectively. The inhibition by baclofen and GABA could be reversed by a depolarizing prepulse to +80 mV, whereas the effect of the α-conotoxins was slower and unaffected by a prepulse, suggesting the involvement of a voltage-independent pathway. Taken together, HEK293 cells provide a suitable expression system to study $GABA_BR$ modulation of $Ca_v2.2$ channels and confirm the role of $GABA_BRs$ in mediating the effects of analgesic α-conotoxins.
1. Callaghan et al. (2008) *J. Neurosci*. **28:**10943-51.

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RGS Proteins Maintain Robustness of GPCR-GIRK Coupling
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Regulators of G-protein signaling (RGS) are GTPase activating proteins (GAP) that reduce response amplitudes of activated G-protein coupled receptors (GPCRs). We discovered that, although RGS proteins drastically accelerate kinetics of GPCR-coupled K+ currents (GIRK), they actually increased amplitudes of inhibitory neurotransmitter-evoked GIRK currents. The RGS-Box domain alone is sufficient for stimulation of transmitter activation of K+ currents, but its membrane association enhances the efficiency of stimulation. Moreover, RGS4 mutants with compromised GAP activity still augment GPCR-GIRK coupling. Among the pertussis toxin sensitive G-proteins, we found that RGS4 selectively stimulates G alpha-o to maintain robustness of GPCR-GIRK coupling. Opposing actions of RGS proteins thus both stimulate and inhibit G-proteins to modulate ultimate amplitudes of transmitter-induced GIRK currents and to differentiate signal intensity coupled to various G-protein isoforms.

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Active Site Hydration and Water Diffusion in Cytochrome P450Cam: A Highly Dynamic Process
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Cytochrome P450s are essential hemoprotein monooxygenases that catalyze a variety of biochemical processes including drug metabolism, lipid and steroid biosynthesis, and degradation of pollutants. As an enzyme from Pseudomonas putida that catalyzes the regio- and stereo-specific hydroxylation of camphor, cytochrome P450cam has long served as a model system for studying P450s. Water molecules are known to play an important role in the enzymatic activity and must be able to enter and exit the active site of P450cam. Here long-timescale molecular dynamics (MD) simulations (300 ns) are performed on both the apo- and camphor-bound P450cam. Water diffusion into and out of